Guidelines for Zebrafish Satellite Facilities

Purpose:

This document outlines the minimum requirements for maintaining a zebrafish satellite facility in the NIH Intramural Research Program (IRP). The zebrafish is an important research animal model and can be successfully maintained and used in satellite facilities away from a centrally managed animal care facility. Zebrafish facilities can vary in research goals, size, and complexity; however common principles, as described in the *Guide for the Care and Use of Laboratory Animals (Guide)*, underlie all IRP zebrafish facilities and operations. This guideline requirement may be adapted for other IRP aquatic species that are maintained in satellite facilities.

Definitions:

- Animal any live, vertebrate animal used or intended for use in research, research training, experimentation, biological testing or for related purpose. Zebrafish are considered an animal after hatching (i.e., > 72 hours post fertilization^{1,2}).
- Satellite facility (SF) for definition, see <u>Manual Chapter 3040-2 Animal Care and Use in the</u> <u>Intramural research program³ or ARAC Guidelines for ACUC Oversight of Satellite Facilities, Study</u> <u>Areas, Laboratories, and other Animal Activity Area⁴</u>.

Requirements and Responsibilities:

- Satellite Facility Registration The IC Animal Care and Use Committee (ACUC) registers all satellite facilities, to include zebrafish incubators designated as SF, with the Office of Animal Care and Use (OACU) to ensure the space and husbandry program meets regulatory and compliance requirements. The OACU reviews and concurs with IC requests to create and maintain satellite animal holding facilities.
- 2. The Principal Investigator (PI) or their designee is responsible for the day-to-day management of the satellite facility; however, they work with the IC ACUC and veterinary staff to ensure the animals' health and welfare needs are being met.
- Veterinary Care Oversight The IC ACUC designates a veterinarian to oversee the veterinary care
 of the zebrafish held in zebrafish satellite facilities (i.e., incubators). This veterinarian may conduct
 visits at intervals appropriate to programmatic needs or when there are health or welfare
 concerns.
- 4. Room Layout Zebrafish incubators are configured to provide a stable and favorable environment that produces and maintains the health, welfare, and productivity of zebrafish.
- 5. Additional responsibilities may apply to systems or incubators that are used for quarantine or chemical hazards, as determined by the Division of Occupational Health and Safety (DOHS), and as outlined within an approved Animal Study Proposal (ASP) or facility Standard Operating Procedure (SOP).
- 6. Husbandry:
 - a. Zebrafish husbandry should be provided by qualified personnel trained on zebrafish biology, care, euthanasia, and the SOPs applicable to that satellite facility⁵.
 - b. Personnel performing husbandry tasks also require sufficient understanding of the housing system to identify malfunctions and have the appropriate resources to address issues in a timely manner.
 - c. The PI maintains training records for research staff. Contract staff members meet the husbandry training standards and training documentation requirements specified in their contract.

- Satellite animal care documentation and equipment monitoring should be maintained in some form for all satellite facilities⁵, though the parameters and frequency of environmental monitoring may vary. All records should be available upon IC ACUC or veterinarian request.
- 8. Incubator signage:
 - a. Incubators that house embryos or fish should be clearly marked (i.e., "Zebrafish Incubator") for clear identification during inspections.
 - b. Emergency contacts with at least two contact numbers (including the IC veterinarian) should be conspicuously posted on or in the immediate area of the incubator.
 - c. Incubator set temperature and acceptable range. Incubators should be maintained in the range of 26-30°C (78.8-86.0°F) unless otherwise described in an approved ASP.
 - d. Incubator light cycle (if applicable)⁶.
 - e. When applicable, signage indicating ABSL 2 or chemical hazards, should be posted as per ASP.
- 9. Responsibilities for zebrafish incubators:
 - a. Daily checks of the following should occur when animals are present⁵:
 - i. Incubator has power and temperature is reported to be within proper range.
 - ii. Larval housing (e.g., petri dishes) are all stable and have not been spilled or knocked over.
 - iii. Larval housing or secondary container (e.g., holding trays) are marked with a lab (if a shared incubator), date of fertilization, and initials of the investigator responsible for the animals.
 - iv. Gross die-off of embryos or larvae are removed.
 - v. No fish are 7+ dpf (unless described in ASP).
 - vi. Within satellite facilities, the PI or their designee should document animal checks and note gaps in the incubator use via the IC satellite animal care documentation. The incubator care forms are maintained by investigator staff and should be able to show records when asked.
 - b. Yearly responsibilities:
 - i. Animal care documentation should be maintained in accordance to IC and NIH record management requirement.
 - ii. IC personnel may verify the temperature in zebrafish incubators annually via a separate thermometer or probe.
- 10. Disaster Planning Disaster plans and support mechanisms for zebrafish satellite facilities should address preparations and responses to events such as staff shortages, electrical and heat outages, and fires^{3,7}.

References:

- 1. NIH ARAC Guidelines for Animal Study Proposals uing of Zebrafish in the NIH Intramural Research Program. (NIH OACU, 2023).
- 2. Public Health Service Policy on Humane Care and Use of Laboratory Animals. (ed. National Institute of Heath, O.o.L.A.W.) (2015).
- 3. 3040-2 Animal Care and Use in the Intramural Research Program. (ed. Assessment, O.o.m.) (NIH Policy Manual, 2023).
- 4. NIH ARAC Guidelines for ACUC Oversight of Satellite Facilites, Study Areas, Laboratories, and other Animal Activity Areas. (NIH OACU, 2023).
- 5. National Research Council Committee for the Update of the Guide for the, C. & Use of Laboratory, A. The National Academies Collection: Reports funded by National Institutes of

Health. in *Guide for the Care and Use of Laboratory Animals* (National Academies Press (US) Copyright © 2011, National Academy of Sciences., Washington (DC), 2011).

- 6. Zynda, J.R. Chapter 25 Aquatics Facility Design Considerations: Incorporating Aquatics into an Animal Facility. in *The Zebrafish in Biomedical Research* (eds. Cartner, S.C., *et al.*) 265-277 (Academic Press, 2020).
- 7. Frederickson, S.C., Pridgen, W. Ginty, I. Clark, T.S. . Incubator Management for Investigators and IACUCs. *LAS Pro*, 34-36 (2023).

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